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Chemical Abstracts, 8th Collective Index, Subject Index p 7184S Entry: Cholest-5-en-3β-ol, 25-methyl-acetate, *75* 64098u
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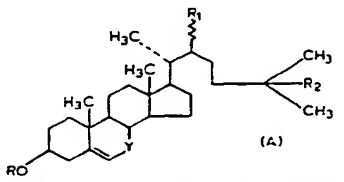
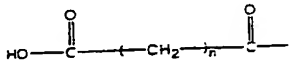
(54) 25-Alkyl Cholesterol Derivatives

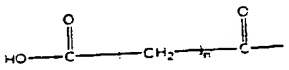
(57) Compounds of formula (A):



wherein R_2 is an alkyl group having from 1 to 4 carbon atoms, R is a hydrogen atom, an alkanoyl group having from 2 to 7 carbon atoms, or a radical of the formula:



in which *n* is a positive integer less than 4, Y is a methylene or carbonyl group, provided that when Y is a carbonyl group, R_1 is a hydrogen atom, and when Y is a methylene group, R_1 is the group OX in which X is a hydrogen atom, an alkanoyl group having from 2 to 7 carbon atoms, or a radical of the formula:



in which *n* is a positive integer less than 4, and the wavy line represents *R* or *S* stereochemistry, processes for their preparation, pharmaceutical and veterinary formulations containing them, and their uses as inhibitors of the activity of HMGCoA reductase and as antiprotozoal agents.

Certain of the chemical formulae appearing in the printed specification were submitted in formal form after the date of filing.

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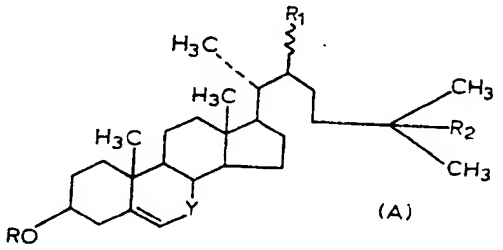
SPECIFICATION

25-Alkylcholesterol Derivatives

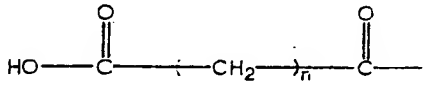
The present invention relates to 25-alkylcholesterol derivatives, to processes for their preparation, to pharmaceutical and veterinary formulations containing them and to their uses as inhibitors of the activity of HMG CoA reductase and as antiprotozoal agents.

The present invention provides in one aspect compounds of formula (A)

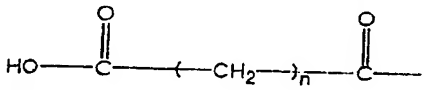


(A)

wherein R_2 is an alkyl group having from 1 to 4 carbon atoms, R is a hydrogen atom or an alkanoyl group having from 2 to 7 carbon atoms or a radical of the formula

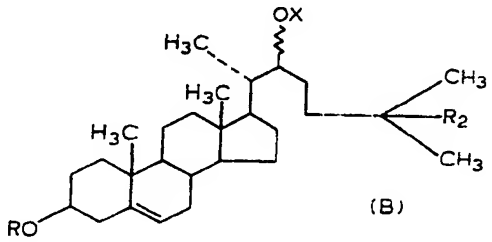


in which n is a positive integer less than 4, Y is a methylene or carbonyl group provided that when Y is a carbonyl group, R_1 is a hydrogen atom, and when Y is a methylene group, R_1 is the group OX in which X is a hydrogen atom, an alkanoyl group having from 2 to 7 carbon atoms, or a radical of the formula



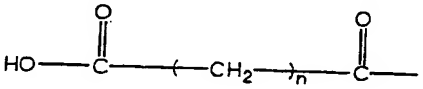
in which n is a positive integer less than 4; and the wavy line represents R or S stereochemistry.

An embodiment of the present invention are compounds of formula (B)



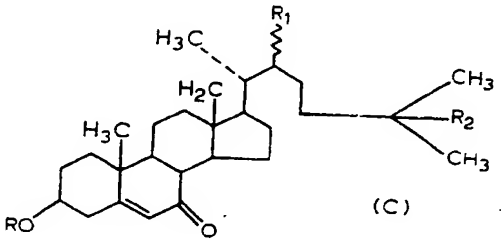
(B)

wherein R_2 is an alkyl group having from 1 to 4 carbon atoms R and X are the same or different and each is a hydrogen atom, an alkanoyl group having from 2 to 7 carbon atoms or a radical of the formula



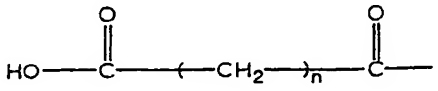
in which n represents a positive integer less than 4; and the wavy lins represents R or S stereochemistry.

Another embodiment of this invention are compounds of formula (C)



(C)

wherein R_2 is an alkyl group having from 1 to 4 carbon atoms, R_1 is a hydrogen atom, R is a hydrogen atom, an alkanoyl group having from 2 to 7 carbon atoms or a radical of the formula



in which n represents a positive integer less than 4, and the wavy line represents R or S stereochemistry.

The compounds of the present invention are useful because of their valuable pharmacological properties. Thus, for example, they inhibit the activity of β-hydroxy-β-methylglutaryl coenzyme A (HMG CoA) reductase, an enzyme which controls the rate at which cholesterol is synthesized in a mammalian liver (one of the two principal sources of serum cholesterol).

A major risk in the development of atherosclerotic disease and associated clinical conditions is the level of circulating total serum cholesterol. As the level of total serum cholesterol rises above 180 mg/ml the risk of atherosclerosis also increases. The low density lipoproteins which are rich in cholesterol have been implicated as the primary vehicle for carrying cholesterol which is deposited in tissues.

The major source of arterial cholesterol in the atherosclerotic patient appears to be of endogenous origin. A reduction in the rate of cholesterol biosynthesis will lead to a lowering of serum cholesterol levels. The rate limiting step in the biosynthesis of cholesterol is the enzymatic reduction of β-hydroxy-β-methylglutaryl CoA (HMG CoA) to mevalonic acid (MVA) by the enzyme HMG CoA reductase. Therefore, the regulation of cholesterol biosynthesis by suppressing the activity of HMG CoA reductase will lead to a lowering of serum cholesterol levels.

Compounds of the present invention inhibit the activity of HMG CoA reductase, and this type of activity is useful in controlling type II hypercholesterolemia, an inherited condition caused by an autosomal dominant mutation of a single gene locus (Brown and Goldstein, Science *191*, 150 (1976)).

Reduction of HMG CoA reductase activity by the compounds of the present invention is demonstrated by the following assay. Male rats of the CD strain from Charles River weighing 180—250 g., initially being kept on a regular laboratory diet, are used. The rats are maintained in a reverse light cycle room for 3 to 6 days. 20,25-Diazacholesterol is administered for a total of 6 days at a dose of 5 mg/kg/day (IG). In the last 3 days, the test compound is administered together with the 20,25-diazacholesterol, and both compounds are given 2 hours prior to the test on the last day. The rats are anaesthetized with ether, sacrificed, and the livers are then removed. Liver microsomes are collected by differential centrifuguation after homogenation, and are used as the source of the HMG CoA reductase. Details of the assay are reported in L. W. White and H. Rudney, Biochemistry *9*, 2713 (1970); Brown *et al.*, J. Biol. Chem. *248*, 4731 (1973); and C. A. Edwards, Biochem, Biophys. Acta. *409*, 39 (1975). The percent change in formation of [^{14}C]-mevalonic acid from [^{14}C]-HMG CoA is used as a measure of enzyme activity for treated groups versus control groups of rats. If the treated groups have less activity, and the decrease is statistically significant at $P \leq 0.05$, the compound is rated active.

The compounds 25-methylcholest-5-en-3β,22-diol and 3β-hydroxy-25-methylcholest-5-en-7-one of the present invention were found active by the above test at doses of 5 mg/kg (IG). The corresponding compounds of the natural series lacking the 25-methyl group, i.e. cholest-5-en-3β,22-diol and 3β-hydroxycholest-5-en-7-one, were both found inactive by the above test at doses of 30 mg/kg (both IG and SC). The explanation regarding the difference in biological activities between the synthetic and the natural series is probably due to the difference in the rate of metabolism of the two series; see Counsell *et al.*, J. of Lipid Research, *18*, No. 1, 24—31, (1977). These results are surprising in view of M. S. Brown and J. L. Goldstein, J. of Biol, Chem., *249*, No. 22; 7306—7314, 7308 (1974) which stated that the addition of a methyl or an ethyl group at position 24 of cholesterol significantly reduced its HMG CoA reductase inhibitory activity.

The compound 25-methylcholest-5-en-3β-ol-3β-acetate of the present invention has been disclosed in Elser, W. *et al., Mol. Crys. and Liq. Crys. 13*, p. 255—270 (1971). The article teaches the mesomorphic behaviour of modified cholesterols and makes no mention of pharmaceutical utility or pharmaceutical preparations.

The compounds of the present invention may be combined by art recognized techniques with pharmaceutically or veterinary acceptable carriers or diluents to yield novel pharmaceutical or veterinary formulations respectively.

Accordingly, the present invention provides in a further aspect a pharmaceutical formulation which comprises one or more of the compounds of formula (A) together with a pharmaceutically acceptable carrier or diluent.

In another aspect, the present invention provides a veterinary formulation which comprises one or more of the compounds of formula (A) together with a veterinary acceptable carrier or diluent.

The concentration of active ingredient in the formulations is not critical, but is preferably from 1 to 80% by weight. These formulations can be administered orally, suitable forms for such administration including tablets, lozenges, capsules, dragees, pills, powders, solutions, suspensions

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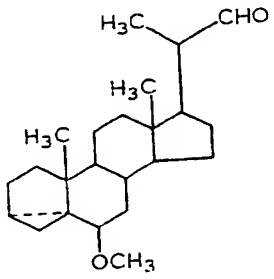
and syrups. Acceptable pharmaceutical carriers are exemplified by gelatin capsules; sugars such as lactose or sucrose; starches such as corn starch or potato starch; cellulose derivatives such as sodium carboxymethyl cellulose, ethyl cellulose, methyl cellulose, or cellulose acetate phthalate; gelatin; talc; calcium phosphates such as dicalcium phosphate or tricalcium phosphate; sodium sulfate; calcium sulfate; polyvinyl pyrrolidone; acacia; polyvinyl alcohol; stearic acid; alkaline earth metal stearates such as magnesium stearate; oils such as peanut oil cottonseed oil, sesame oil; olive oil, corn oil, oil of theobroma; water; agar; alginic acid; and benzyl alcohol, as well as other non-toxic compatible substances normally used in pharmaceutical formuations.

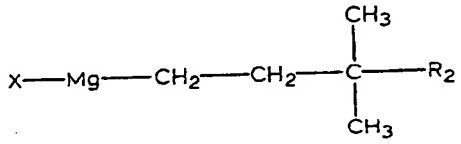
In addition to the aforementioned utility, compounds of the present invention are also useful as antiprotozoal agents, particularly as anti-*Trichomonas vaginalis* agents.

The compounds of the present invention may be obtained by processes orginating with the compound 6β-methoxy-3α, 5-cyclo-5α-23,24-bisnorcholan-22-al [Steriods, *15*, 113 (1970)] having formula (I) as starting material

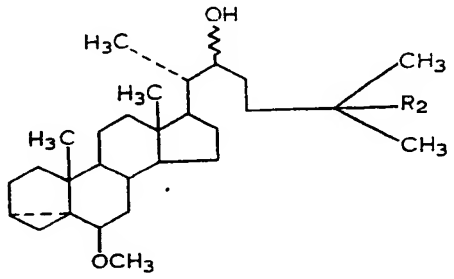


(I)

6β-methoxy-3—,5-cyclo-5α-23,24-bisnorcholan-22-al is alkylated by the addition of a 3,3-dimethylalkyl magnesium halide having the formula

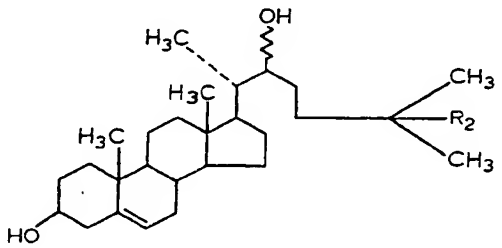


wherein X is a halogen atom and R_2 is an alkyl group having from 1 to 4 carbon atoms. The resultant i-steroid alcohol of formula (II)



(II)

is rearranged by heating with an acid such as 4-methyl-benzensulphonic acid monohydrate in aqueous dioxane, giving rise to a diol falling within the scope of formula (A), having formula (III)



(III)

Heating a compound of formula (II) with an alkanoic acid having from 2 to 7 carbon atoms yields a 3-ester falling within the scope of formula (A) having the formula (IV)

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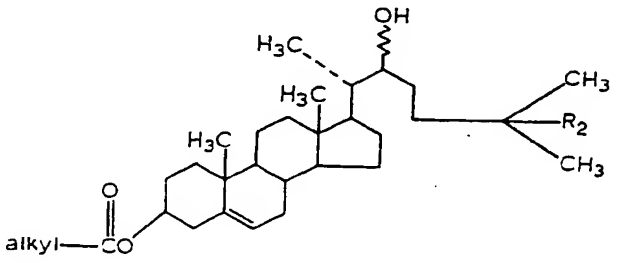
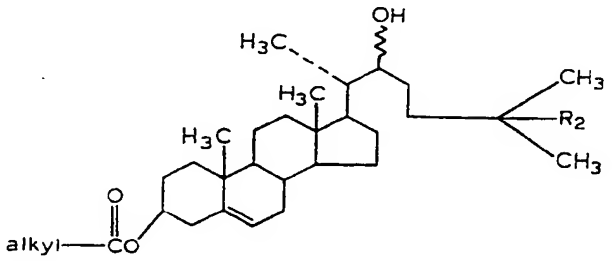


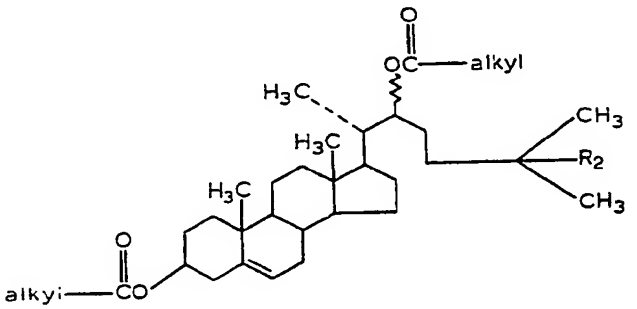
(IV)

whereas heating a compound of formula (III) in pyridine with an alkanoic acid anhydride or chloride yields a mixture of esters falling within the scope of formula (A) having the formulae (V) and (VI)



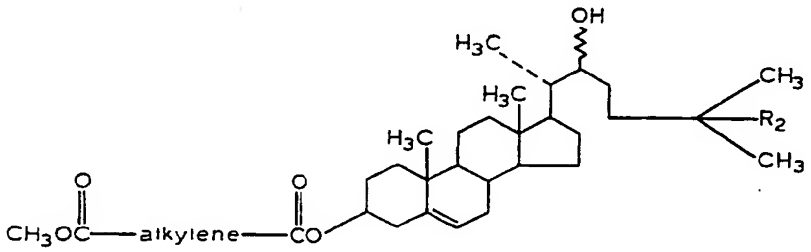
(V)

and

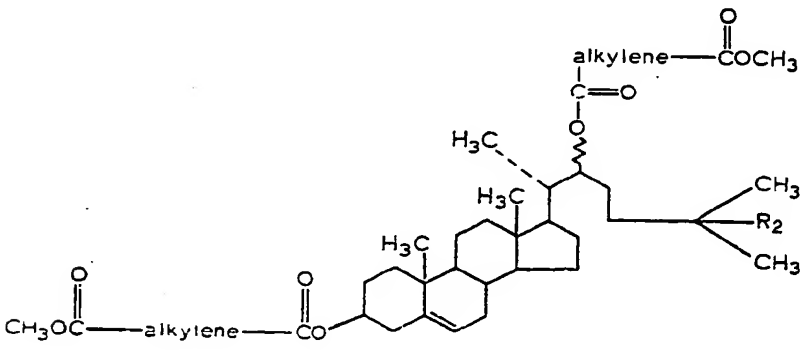


(VI)

which may be separated from each other by chromatography, for example, on silica gel, using eluents such as methyl-benzene and mixtures thereof with increasing amounts of ethyl acetate. In an analogous fashion, heating a compound of formula (III) in pyridine with a methyl Ω-chloro-Ω-oxoalkanoate yields a mixture of esters having the formulae (VII) and (VIII)



(VII)



(VIII)

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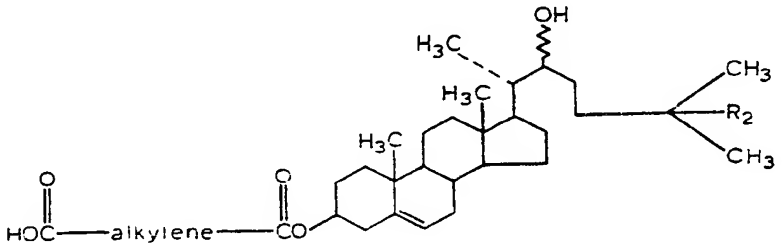
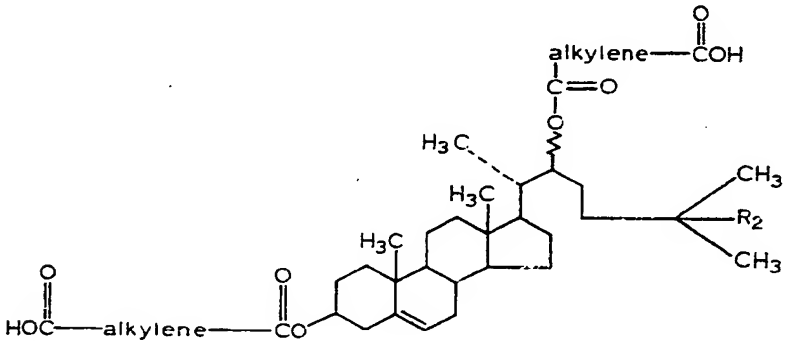
which may be separated from each other by chromatography on, for example, silica gel as aforesaid. Heating an ester of formula (VII) or (VIII) with lithium iodide in pyridine, 2,6-dimethylpyridine, or 2,4,6-trimethylpyridine yields an ester falling within the scope of formula (A) having the formula (IX) or (X)



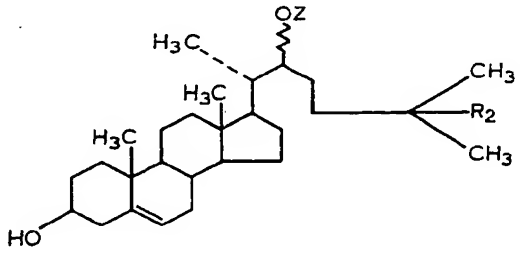
(IX)

or



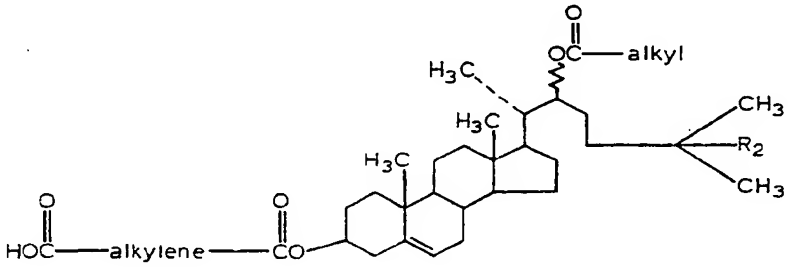
(X)

respectively. Heating a compound of formula (VI) or (X) with sodium bicarbonate in aqueous ethanol affords a 22-ester falling within the scope of formula (A) having the formula (XI)



(XI)

wherein Z is a 1-oxoalkyl or Ω-carboxyl-1-oxoalkyl group, respectively. Finally, heating a compound of formula (IX) in pyridine with an alkanoic acid anhydride or chloride affords an ester falling within the scope of formula (A) and having the formula (XII)



(XII)

As an exception to the foregoing procedure, a compound of formula (IX) in which the esterifying moiety is 3-carboxy-1-oxopropyl is preferably prepared by heating a compound of formula (III) with butanedioic acid anhydride in pyridine. In each of formula (I) to (XII) hereinabove, R_2 represents an alkyl group, preferably containing fewer than 5 carbon atoms, and the wavy line represents R or S stereochemistry.

Optionally, alkylation of the compound of formula (I) may be accomplished by the addition of a 3,3-dimethylalkyl triphenyl phosphonium iodide to give compounds of formula (XIII)

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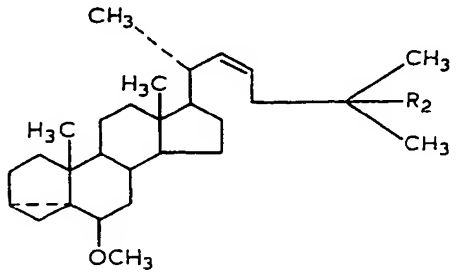
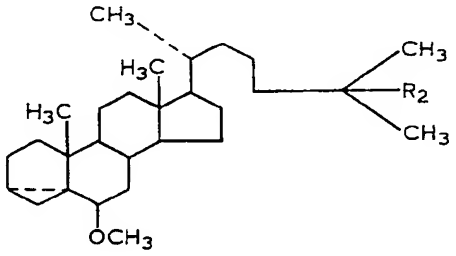


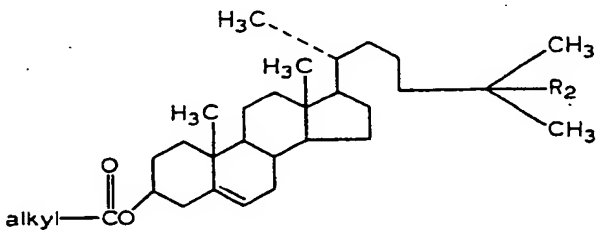
(XIII)

which may be hydrogenated over 5% palladium/carbon to give the compounds (XIV)



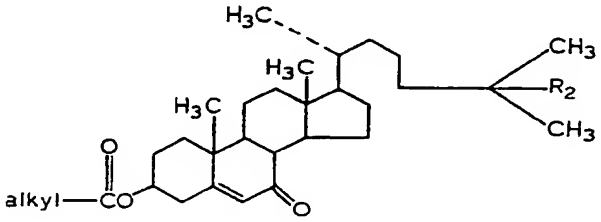
(XIV)

which may be rearranged by heating with an alkanoic acid to give compounds of formula (XV)



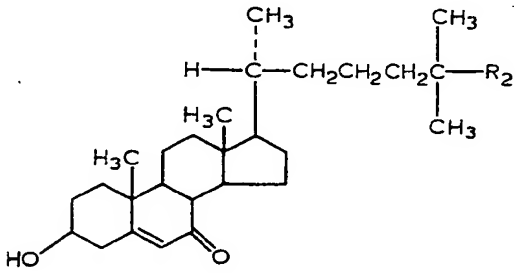
(XV)

compounds of formula (XV) when contacted with chromium oxide and pyridine in dichloromethane under nitrogen afford the corresponding 7-ones which fall within the scope of formula (A), having formula (XVI)



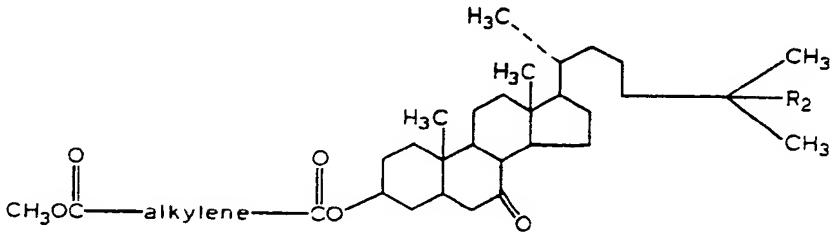
(XVI)

Heating an ester for formula (XVI) with sodium bicarbonate in aqueous ethanol affords the corresponding 3β-hydroxy compounds which fall within the scope of formula (A) having the formula (XVII)



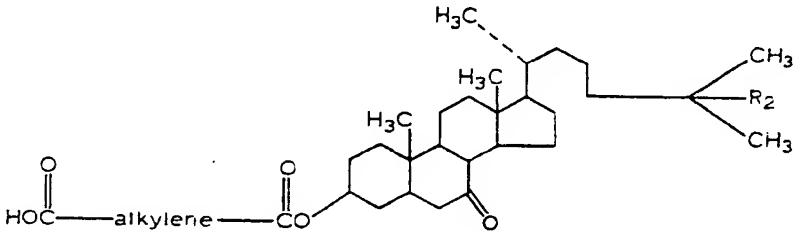
(XVII)

Heating a compound of formula (XVII) in pyridine with a methyl Ω-chloro-Ω-oxoalkanoate and heating the mixed ester of formula (XVIII) thus obtained



(XVIII)

with lithium iodide in pyridine, 2,6-dimethylpyridine, or 2,4,6,-trimethylpyridine affords an ester falling within the scope of formula (A) and having the formula (XIX)



(XIX)

As an exception to the foregoing procedure, a compound of formula (XIX) wherein the esterifying moiety is 3-carboxy-1-oxopropyl is preferably prepared by heating a compound of formula (XVII) with butanedioic acid anhydride in pyridine.

In each of formulae (XIII) to (XIX) herein above R_2 represents alkyl containing 1 to 4 carbon atoms, and the wavy line represents R or S stereochemistry.

The following Examples described in detail compounds illustrative of the present invention and methods which have been devised for their preparation. Throughout the Examples hereinafter set forth, temperatures are given in degrees centigrade (°C) and relative amounts of materials in parts by weight unless part by volume is specified. The relationship between parts by weight and parts by volume is the same as that existing between grams and milliliters.

Example 1

50 Parts by volume of 3,3-dimethylbutyl magnesium chloride solution 1.35 M was added dropwise to a solution of 13.4 parts of 6β-methoxy-3α, 5-cyclo-5α-23,24-bisnorcholan-22-al in 100 parts by volume of tetrahydrofuran under nitrogen at a temperature of 0 to 5°C. After the addition was completed, the cooling bath was removed and the reaction mixture was stirred at room temperature for about one hour. 100 parts by volume of saturated ammonium chloride solution was then added to the mixture. 100 parts by volume of ether was subsequently added to the hydrolyzed reaction solution, and the layers were separated. The aqueous phase was extracted with an additional portion of ether and the combined extracts were washed with saturated salt solution and dried over sodium sulphate. Removal of the solvent gave the product 6β-methoxy-25-methyl-3α,5-cyclo-5α-cholestan-22-ol as an oil which was used in its crude form for subsequent reactions.

Example 2

17.2 Parts of the compound prepared in Example 1 was added to 75 parts by volume of glacial acetic acid and the mixture was heated on a steam bath for 3 hours. The mixture was then cooled and 300 parts by volume water was added, forming an oily solid. This oily solid was extracted using ether and the ethereal extracts were washed with 5% sodium bicaronate solutions until the aqueous extracts remained basic. The organic phase was then dried over sodium sulphate and the solvent was removed. The solid product was recrystallized from acetone to give the product in two crops. Chromatography of the mother liquors provided an additional portion of the desired product. An additional recrystallization from acetone gave the analytical compound 25-methylcholest-5-ene-3β,22-diol-3 acetate which melts at 185 to 187°C.

Example 3

0.1 Part tosyl acid hydrate was added to a solution of 2.5 parts 6β-methoxy-25-methyl-3α,5-cyclo-5α-cholestan-22-ol in 60 parts by volume dioxane and 20 parts by volume water, and the reaction mixture was heated on a steam bath for 5 hours. Water was then added to the reaction mixture and the solution was extracted with ether. The combined ether extracts were washed with a saturated salt solution and dried over sodium sulphate. The solvent was removed to yield an oil, which upon trituration with ethanol afforded a solid product. Recrystallization from aqueous ethanol afforded the pure product 25-methylcholest-5-ene-3β,22-diol which melts at 191 to 193°C. Alternatively, 25-methylcholest-5-ene-3β,22-diol was prepared in the following manner. 5 parts by volume of 5%

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sodium hydroxide was added to a solution of 1.0 part 25-methylcholest-5-ene-3β, 22-diol-3-acetate in 20 parts by volume ethanol and the reaction mixture was refluxed for 1½ hours. The hot reaction mixture was filtered and a small amount of water was added to the filtrate. Upon cooling, the product resulted in two crops. Recrystallization from ethanol gave the pure compound melting at 187 to 191°C.

Example 4

To 1.6 parts 25-methylcholest-5-ene-3β,22-diol in 25 parts by volume pyridine was added 1.0 part succinic anhydride and the reaction mixture was heated on the steam bath for 18 hours. Water was then added to the cooled reaction mixture and an oil separated, which solidified upon stirring. The solid was recrystallized from aqueous ethanol and then ethyl acetate/hexane to afford 25-methylcholest-5-ene-3β,22-diol-3β, hemisuccinate which melts at 190 to 193°C.

Example 5

To 2.5 parts 25-methylcholest-5-ene-3β,22-diol-3β-acetate in 20 parts by volume pyridine was added 10 parts by volume acetic anhydride, and the reaction mixture was left standing at room temperature for 5 hours. The mixture was then warmed on a steam bath for one hour and after cooling, 200 parts by volume of water was added to said mixture. The solid which formed was recrystallized from ethanol to give 25-methylcholest-5-en-3β,22-diol-3β,22-diacetate which melts at 175 to 177°C.

Example 6

To 200 parts by volume methylene chloride containing 15.8 parts pyridine under a nitrogen atmosphere was added in portions, at room temperature, 10 parts chromic acid, which had been previously dried over phosphorous pentoxide. The burgundy coloured solution was stirred for 30 minutes and then 2.7 parts 25-methylcholest-5-ene-3β,22-diol-3β,22-diacetate in 15 parts by volume methylene chloride was added rapidly. The reaction mixture was stirred at room temperature for 20 hours and was then decanted from the insoluble residue. The residue was washed with several portions of ether, and the ether washings were combined with the decantate. The ethereal-decantate solution was treated with Florex and subsequently filtered through diatomaceous earth. The filtrate was washed with 1 N hydrochloric acid and then saturated salt solution, dried over sodium sulphate, treated with activated charcoal and then filtered. The solvent was removed, and the crude solid product was recrystallized from ethanol to afford the pure compound 3β,22-dihydroxy-25-methylcholest-5-ene-7-one 3β,22-diacetate melting at 260 to 262°C.

Example 7

To 1.3 parts 3β,22-dihydroxy-25-methylcholest-5-ene-7-one-3β,22-diacetate in 130 parts by volume ethanol was added 13 parts by volume of 5% sodium bicarbonate solution and the reaction mixture was refluxed for 2.5 hours. After cooling, the orange solution was acidified with a small amount of acetic acid, and 200 parts by volume of water was added. The precipitate which formed was recrystallized from ethanol in two crops. These were combined and recrystallized twice from an ether/hexane mixture to afford 3β,22-dihydroxy-25-methylcholest-5-en-7-one-22-acetate melting at 217 to 218°C.

Example 8

5.0 Parts by volume of 5% sodium bicarbonate solution was added to 50 parts by volume ethanol containing 1.0 part 25-methylcholest-5-ene-3β,22-diol-3β,22-diacetate and the mixture was refluxed for 2.5 hours. Water was added and the solid product formed was collected and recrystallized from ethanol to afford 25-methylcholest-5-ene-3β,22-diol-22-acetate which melts at 210 to 211.5°C.

Example 9

To 0.4 parts of 25-methylcholest-5-ene-3β,22-diol-3β-acetate suspended in 15 parts by volume acetone at room temperature was added 0.35 parts of Jones reagent and the reaction mixture was stirred for 30 minutes. The excess reagent was then destroyed using a small amount of isopropanol and the reaction mixture was decanted from the insoluble residue. Addition of water to the decantate caused formation of a solid which was collected and recrysallized from ethanol to afford 3β-hydroxy-25-methyl-cholest-5-en-22-one-3β-acetate which melts at 187 to 187.5°C.

Example 10

To 1.3 parts 3β-hydroxy-25-methylcholest-5-en-22-one-3β-acetate in 40 parts by volume ethylene glycol was added 7.0 parts hydrazine hydrate (65% hydrazine) and 10 parts by volume ethanol. The reaction mixture was refluxed for 1 hour, after which time it became homogeneous. 3.0 Parts potassium hydroxide pellets were added and the solution was heated to 190°C, and maintained at that temperature for 16 hours, under an atmosphere of nitrogen. Another 3.0 parts potassium hydroxide was then added, and heating was continued for another 24 hours. Water was added to the

cooled solution and the precipitate was collected. The precipitate was dissolved in 1:1; ether/pentane solution and the solution obtained was filtered through a cake of magnesium sulphate. The solvent was removed from the filtrate and the solid product was recrystallized from acetone and then methanol, to afford 25-methylcholest-5-en-3β-ol which melts at 153 to 156°C.

Example 11

To 0.9 part 25-methylcholest-5-en-3β-ol in 12 parts by volume pyridine was added 5.0 parts by volume acetic anhydride and the reaction mixture was allowed to stand for 16 hours. Water was then added and the precipitate which was collected was recrystallized from methanol to afford 25-methylcholest-5-en-3β-ol-3β-acetate which melts at 146 to 148°C.

Example 12

To 70 Parts by volume of methylene chloride containing 7.8 parts pyridine under a nitrogen atmosphere was added 5.0 parts chromic oxide, in portion, at room temperature. After stirring the burgundy coloured solution for half an hour, 1.0 part of 25-methycholest-5-en-3β-ol-3β-acetate in 5 parts by volume methylene chloride was added and the mixture was stirred at room temperature for 16 hours. Three volumes of ether were then added to the reaction mixture and the solution was decanted from the insoluble residue. Florex was added to the solution, which was then filtered through diatomaceous earth. The filtrate was washed with 1 N hydrochloric acid, followed by 5% sodium bicarbonate, and then a saturated salt solution. The solution was dried over sodium sulphate and the solvent was removed to give an oily residue. Crystallization from ethanol containing a slight amount of water afforded 3β-hydroxy-25-methycholest-5-en-7-one-3β-acetate which melts at 183 to 186°C.

Example 13

To 0.48 parts of 3β-hydroxy-25-methylcholest-5-en-7-one-3β-acetate in 40 parts by volume ethanol was added 4.0 parts by volume 5% sodium bicarbonate solution and the reaction mixture was refluxed for 3 hours. After cooling, a small amount of acetic acid was added to neutralize the base, followed by the addition of 50 parts by volume of water which caused a precipitate to form. The precipitate was collected and chromatographed over Woelm silica gel using ethyl acetate in benzene as the eluent. Recrystallization from aqueous ethanol afforded 3β-hydroxy-25-methylcholest-5-en-7-one which melts at 176 to 178°C.

Example 14

To 0.2 parts of 3β-hydroxy-25-methylcholest-5-en-7-one in 6 parts by volume pyridine was added 0.5 part succinic anhydride and the reaction mixture was heated on a steam bath for 18 hours. Water was added to the cooled reaction mixture, followed by the addition of 1 N hydrochloric acid. The solid was collected and dissolved in ether. The ethereal solution was washed with 1 N hydrochloric acid, saturated salt solution, and then dried over sodium sulphate and filtered. The solvent was removed and the product was recrystallized from methanol to afford 3β-hydroxy-25-methylcholest-5-en-7-one-3β-hemisuccinate which melts at 211 to 212°C.

Example 15

To 7.8 parts 3,3-dimethylbutyltriphenyl phosphonium iodide in 75 parts by volume tetrahydrofuran under a nitrogen atmosphere was added 9.4 parts by volume of 1.75 M phenyllithium solution over a 5 minute period, and the orange-red solution was stirred at room temperature for 15 minutes. After cooling the solution to —70°C, 4.0 parts 6β-methoxy-3α,5-cyclo-5α-23,24-bis-norcholan-22-al in 25 parts by volume tetrahydrofuran was added over a 5 minute period and the reaction mixture was stirred at —70°C for 30 minutes before removing the cooling bath and letting the reaction warm to room temperature. Saturated ammonium chloride solution was then added, followed by the addition of ether, and the layers were separated. The aqueous phase was extracted with additional ether and the combined extracts were washed with a saturated salt solution and dried over sodium sulphate. Removal of the solvent gave an oil which was taken up in pentane, and after stirring for several minutes at room temperature the precipitate which formed was removed by filtration. The volume of the filtrate was reduced to one-half of the original and the precipitate which again formed was removed by filtration. The precipitate was triphenylphosphene oxide. Removal of the solvent from the filtrate gave the product 6β-methoxy-25-methyl-3α,5-cyclo-5α-cholest-22-ene as an oil, which was used in its crude form for subsequent reactions.

Example 16

4.8 parts of 6β-methoxy-25-methyl-3α,5-cyclo-5α-cholest-22-ene in 200 parts by volume ethanol was hydrogenated at a pressure of 35 psi at room temperature with 4.8 parts 5% palladium/carbon until an equivalent of hydrogen was consumed. The catalyst was removed from the solution by filtration, and the solvent was removed from the filtrate to give the product, 6β-methoxy-25-methyl-3α,5-cyclo-5α-cholestane.

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Example 17

4.3 parts crude 6β-methoxy-25-methyl-3α,5-cyclo-5α-cholestane in 30 parts by volume glacial acetic acid was heated on a steam bath for 2 hours. 30 Parts methanol was added to the solution and, upon cooling, a crystalline product was formed. The solvent was formed and the product was recrystallized from ether/methanol to afford the pure product 25-methylcholest-5-en-3β-ol-3β-acetate which melts at 152/153°C.

Example 18

To a solution of 4 parts 25-methycholest-5-ene-3β,22-diol in 25 parts of pyridine was added a solution of 1 part of acetyl chloride in 5 parts of pyridine. The resultant mixture was stirred at room temperature for 6 hours, and then diluted with an equal volume of water. The mixture thus obtained was extracted with ether, and the extract was consecutively washed with 5% hydrochloric acid and water, then stripped of solvent by vacuum distillation. The residue was taken upon in methylbenzene, and the methylbenzene solution was chromatographed on silic gel, using methylbenzene and mixtures thereof with increasing amounts of ethyl acetate as developing solvents. From eluates selected via thin layer chromatography and stripped of solvent by vacuum distillation, 25-methylcholest-5-ene-3β,22-diol 3-acetate and 25-methylcholest-5-ene-3β,22-diol 3,22-diacetate were isolated as residues which, when recrystallized from ethanol, melt at 185 to 187° and 175 to 177° respectively. (The diester, being less polar, was eluted first).

Example 19

A). To a solution of 4 parts of 25-methylcholest-5-ene-3β,22-diol in 25 parts of pyridine was added a solution of 2 parts of 3-methoxy-3-oxopropanoyl chloride in 5 parts of pyridine. The resultant mixture was stirred at 90 to 95° for 3 hours, and then diluted with an equal volume of water. The mixture thus obtained was extracted with ether, and the extract was consecutively washed with 5% hydrochloric acid and water, then stripped of solvent by vacuum distillation. The residue was taken up in methylbenzene, and the methylbenzene solution was chromatographed on silica gel, using methylbenzene and mixtures thereof with increasing amounts of ethyl acetate as developing solvents. From eluates selected via thin layer chromatography and stripped of solvent by vacuum distallation, 25-methylcholest-5-ene-3β,22-diol 3-(3-methoxy-3-oxopropanoate) and 3,22-bis(3-methoxy-3-oxopropanoate) were isolated as the residues. (The diester, being less polar, was eluted first).

B). A mixture of 4 parts of 25-methylcholest-5-ene-3β,22-diol 3,22-bis(3-methoxy-3-oxopropanoate), 12 parts of lithium iodide, and 30 parts of 2,6-dimethylpyridine was heated at the boiling point under reflux with stirring overnight, whereupon an equal volume of water was added and the solid precipitate which resulted was filtered off, washed with water, and dried in air. The product thus isolated was 25-methylcholest-5-ene-3β,22-diol 3-(hydrogen propanedioate).

Example 20

A mixture of 1 part of 25-methylcholest-5-ene-3β,22-diol-3β-(hemisuccinate), 10 parts of pyridine, and 5 parts of acetic anhydride was stirred at room temperature overnight, then diluted with an equal volume of water. The solid precipitate which resulted was filtered off, washed with water, and dried in air. The product thus isolated was 25-methylcholest-5-ene-3β,22-diol 22-acetate-3β-hemisuccinate.

Example 21

A). To a solution of 4 parts of 3β-hydroxy-25-methylcholest-5-en-7-one in 25 parts of pyridine was added a solution of 2 parts of 3-methoxy-3-oxopropanoyl chloride in 5 parts of pyridine. The resultant mixture was stirred at 90 to 95° for 3 hours, then diluted with an equal volume of water. The mixture thus obtained was extracted with ether, and the extract was consecutively washed with 5% hydrochloric acid and water then stripped of solvent by vacuum distillation. The residue was 3β-hydroxy-25-methylcholest-5-en-7-one 3-(3-methoxy-3-oxopropanoate).

B). A mixture of 4 parts of 3β-hydroxy-25-methylcholest-5-en-7-one 3-(3-methoxy-3-oxopropanoate), 12 parts of lithium iodide, and 30 parts of 2,6-dimethylpyridine was heated at boiling point under reflux with stirring overnight, whereupon an equal volume of water was added and the solid precipitate which resulted was filtered off, washed with water, and dried in air. The product thus isolated was 3β-hydroxy-25-methylcholest-5-en-7-one-3-(3-hydrogen propanedioate).

Example 22

Pharmaceutical formulations were prepared in the following manner with amounts indicating the relative amounts per 1000 tablets, capsules, suppositories or parenteral product.

Tablets

500 Grams of 25-methylcholest-5-ene-3β,22-diol was combined with 265 grams of lactose, 200 grams of corn starch, and 30 grams of polyvinylpyrrolidone, mixed thoroughly, and passed through a 40 mesh screen. The mixture was then granulated with isopropyl alcohol, spread on trays, and dried

at 49°C for 16 hours. The dried granulate was then screened. The granules were mixed thoroughly with 5 grams of magnesium stearate and the mixture compressed into tablets of the appropriate size. There was thus obtained a bath of 1000 tablets having a concentration of active ingredients of 500 mg/tablet.

Capsules

500 Grams of 25-methylcholest-5-ene-3β,22-diol was mixed thoroughly with 87.5 grams of corn starch and 87.5 grams of lactose, passed through a 40 mesh screen, and remixed. 75 Grams of talc was added and the mixture was thoroughly mixed and placed into a No. 00 size hard gelatin capsule by hand or machine using 750 mg. fill per capsule to give a final product containing 500 mg. of active ingredient per capsule.

In the preparation of tablets and capsules from the compounds of the present invention, a variety of excipients can be used. These are summarized as follows: Sugars such as lactose, sucrose, mannitol, or sorbitol; starches such as corn starch, tapioca starch, or potato starch; cellulose derivatives such as sodium carboxymethyl cellulose, ethyl cellulose, or methyl cellulose; gelatin, calcium phosphates such as dicalcium phosphate or triacalcium phosphate; sodium sulfate, calcium sulphate; polyvinylpyrrolidone; polyvinyl alcohol; stearic acid; alkaline earth metal stearates such as magnesium stearate; stearic acid vegetable oils such as peanut oil, cottonseed oil, sesame oil, olive oil, corn oil, surfactants (nonionic, cationic, anionic); ethylene glycol polymers; beta-cyclodextrin; fatty alcohols; hydrolyzed cereal solids; as well as other non-toxic compatible fillers, binders, disintegrants, and lubricants commonly used in pharmaceutical formulations.

Suppositories

500 Grams of cocoa butter was melted in a water or stream bath to avoid local overheating, and then 500 grams of 25-methylcholest-5-ene-3β, 22-diol was either emulsified or suspended in the melt. Finally the mass was poured into cooled metal moulds, which were chrome plated, and the suppository was readily solidified. There was thus obtained a batch of 1000 suppositories having a concentration of active ingredient of 500 mg. per suppository.

In the preparation of suppositories from compounds of the present invention a variety of vehicles and bases can be used. These are summarized as follows: triglycerides of oleic, palmitric, and stearic acids (Cocoa butter), partially hydrogenated cottonseed oil, branched saturated fatty alcohols such as suppository base G, hydrogenated coconut oil triglycerides of C_{12} to C_{18} fatty acids, water dispersible vehicles such as the polyethylene glycols, glycerin, gelatin, polyoxyl 40 stearates, and polyethylene-4-sorbitan monostearates, and materials which can raise the melting point of the suppository base, such as beeswax, and spermaceti.

Parenteral Products

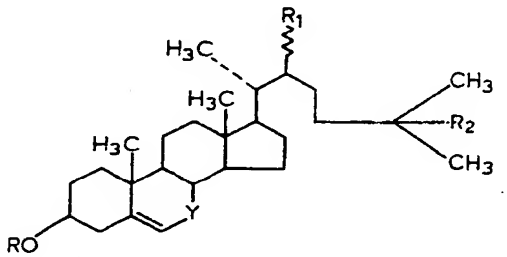
10 Grams of 25-methylcholest-5-ene-3β, 22-diol was dissolved in 1000 milliliters of ethyl alcohol and sesame oil was added to give a total volume of 5000 milliliters. The mixture was filtered and placed into ampoules which were then sealed. The ampoules were then sterilized using an appropriate procedure. There was thus obtained a batch of 1000 ampoules having a concentration of active ingredient of 10 mg/5 ml. per ampoule.

In the preparation of parenteral products from the compounds of the present invention a variety of vehicles and solubilizers can be used. These are summarized as follows: vegetable oils such as peanut, corn, cottonseed, sesame oil, benzyl alcohol, saline, phosphate buffer, water, ethylene glycol polymers, urea, dimethyl acetamide, triton, dioxolanes, ethyl carbonate, ethyl lactate, glycerol formal, isopropyl myristate, surfactants (nonionic, cationic or anionic), polyalcohols, and ethanol.

It will be understood that any of the compounds of the present invention may be used to produce pharmaceutical formulations in a fashion analogous to that described in Example 22.

Claims

1. A compound of formula (A):



(A)

wherein R_2 is an alkyl group having from 1 to 4 carbon atoms, R is a hydrogen atom an alkanoyl group

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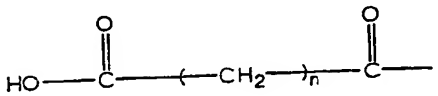
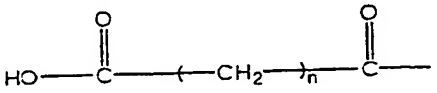
having from 2 to 7 carbon atoms or a radical of the formula



in which n is a positive integer less than 4, Y is a methylene or carbonyl group, provided that when Y is a carbonyl group, R_1 is a hydrogen atom, and when Y is a methylene group, R_1 is the group OX in which X is a hydrogen atom, an alkanoyl group having from 2 to 7 carbon atoms or a radical of the formula:



in which n is a positive integer less than 4, and the wavy line represents R or S stereochemistry.

2. A compound as claimed in claim 1 wherein Y is methylene group, R_1 is a group OX and R_1 R_2 the wavy line and X are as defined in claim 1.

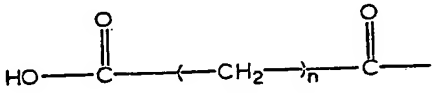
3. A compound as claimed in claim 1 wherein Y is a carbonyl group, R_1 is a hydrogen atom and the wavy line, R, and R_2 are as defined in claim 1.

4. A compound as claimed in claim 1 wherein Y is a methylene group, R is a hydrogen atom, R_1 is a hydroxyl group and R_2 and the wavy line are as defined in claim 1.

5. A compound as claimed in claim 1 wherein Y is a methylene group, R_1 is a hydroxyl group, R is an alkanoyl group having from 2 to 7 carbon atoms and R_2 and the wavy line are as defined in claim 1.

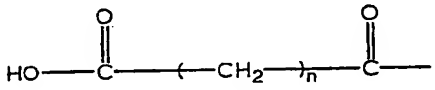
6. A compound as claimed in claim 1 wherein Y is a methylene group, R_1 is a group OX in which X is an alkanoyl group having from 2 to 7 carbon atoms, R is an alkanoyl group having from 2 to 7 carbon atoms and the wavy line and R_2 are as defined in claim 1.

7. A compound as claimed in claim 1 wherein Y is a methylene group, R_1 is a hydroxyl group, R is a radical of the formula:

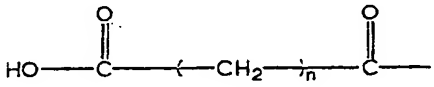


In which n is a positive integer less than 4, and R_2 and the wavy line are as defined in claim 1.

8. A compound as claimed in claim 1 wherein Y is a methylene group, R_1 is a group OX in which X is a radical of the formula:



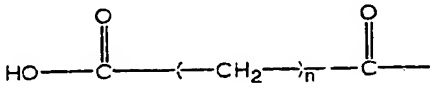
in which n is a positive integer less than 4, R is a radical of the formula:



in which n is a positive integer less than 4, and R_2 and the wavy line are as defined in claim 1.

9. A compound as claimed in claim 1 wherein Y is a methylene group, R_1 is an Ω-carboxyl-1-oxoalkyl group or a 1-oxoalkyl group, R is a hydrogen atom and R_2 and the wavy line are as defined in claim 1.

10. A compound as claimed in claim 1 wherein Y is a methylene group, R_1 is a group OX in which X is an alkanoyl group having from 2 to 7 carbon atoms, R is a radical of the formula:



in which n is a positive integer less than 4, and R_2 and the wavy line are as defined in claim 1.

11. A compound as claimed in claim 1 wherein Y is a carbonyl group, R_1 is a hyrogen atom, R is an alkanoyl group having from 2 to 7 carbon atoms and R_2 and the wavy line are as defined in claim 1.

12. A compound as claimed in claim 1 wherein Y is a carbonyl group, R_1 is a hydrogen atom, R is a hydrogen atom and R_2 and the wavy line are as defined in claim 1.

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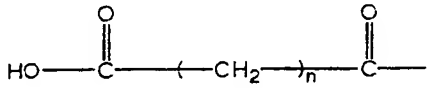
13. A compound as claimed in claim 1 wherein Y is a carbonyl group, R_1 is a hydrogen atom, R is a radical of the formula:



in which n is a positive integer less than 4 and R_2 and the wavy line are as defined in claim 1.

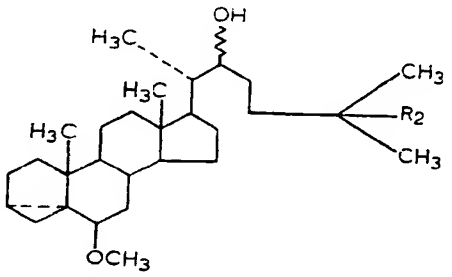
14. 25-methylcholest-5-ene-3β, 22-diol.

15. 25-methylcholest-5-ene-3β, 22-diol-3β-hemi-succinate.

16. 25-methylcholest-5-ene-3β, 22-diol-3-(hydrogen propanedioate).

17. 3β-hydroxy-25-methylcholest-5-ene-7-one-3β-hemi-succinate.

18. A process for the preparation of a compound as claimed in claim 4 which comprises heating a compound of formula (II):

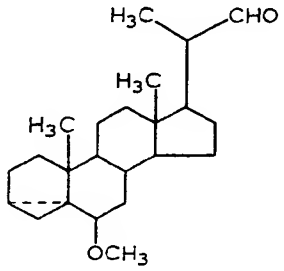


(II)

wherein R_2 and the wavy line are as defined in claim 1 with an acid in aqueous dioxane.

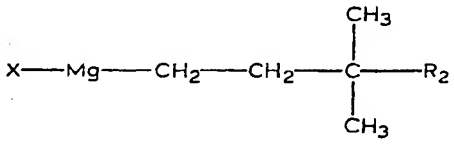
19. A process as claimed in claim 18 wherein the acid is 4-methylbenzenesulphonic acid.

20. A process as claimed in claim 18 or claim 19 wherein the compound of formula (II) is prepared by alkylating the compound of formula (I):



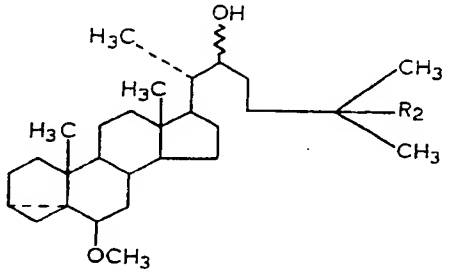
(I)

with a 3,3-dimethyl magnesium halide having the formula:



wherein X is a halogen atom and R_2 is as defined in claim 1.

21. A process for the preparation of a compound as claimed in claim 5 which comprises heating a compound of formula (II):



(II)

wherein R_2 and the wavy line are as defined in claim 1, with an alkanoic acid having from 2 to 7 carbon atoms.

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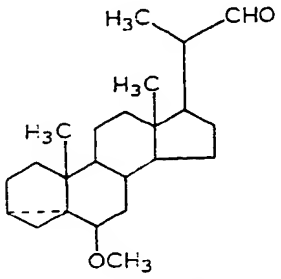
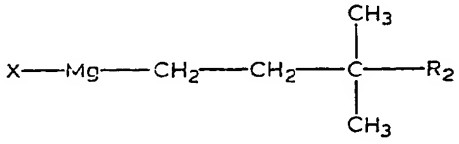
22. A process as claimed in claim 21 wherein the compound of formula (II) is prepared by alkylating the compound of formula (I):



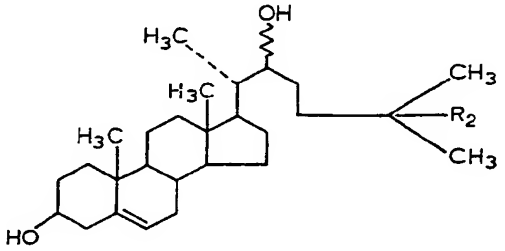
(I)

with a 3,3-dimethyl magnesium halide having the formula



wherein X is a halogen atom and R_2 is as defined in claim 1.

23. A process for the preparation of a compound as claimed in claim 5 which comprises heating a compound of formula (III):

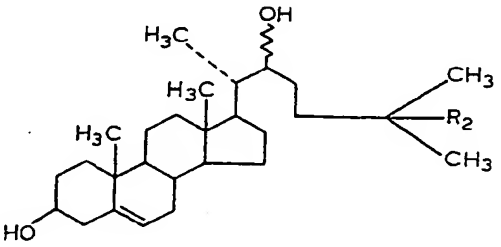


(III)

wherein R_2 and the wavy line are as defined in claim 1, in pyridine with an alkanoic acid anhydride or chloride having from 2 to 7 carbon atoms, and separating the required product from the resulting end products by chromatography.

24. A process as claimed in claim 23 wherein the compound of formula (III) is prepared by a process as claimed in any of claims 18 to 20.

25. A process for the preparation of a compound as claimed in claim 6 which comprises heating a compound of formula (III):



(III)

wherein R_2 and the wavy line are as defined in claim 1, in pyridine with an alkanoic acid anhydride or chloride having from 2 to 7 carbon atoms, and separating the required product from the resulting end products by chromatography.

26. A process as claimed in claim 25 wherein the compound of formula (III) is prepared by a process as claimed in any of claims 18 to 20.

27. A process for the preparation of a compound as claimed in claim 7 which comprises heating a compound of formula (VII)

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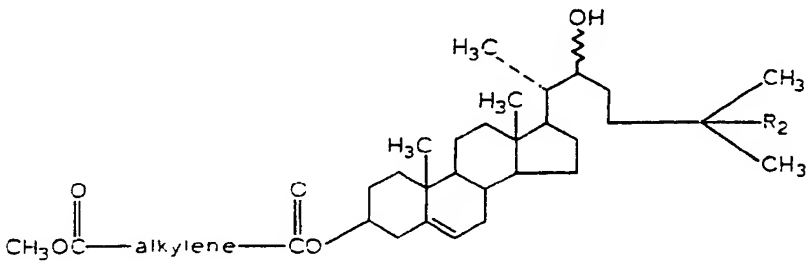
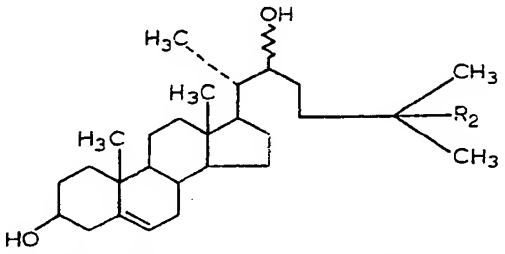


(VII)

wherein R_2 and the wavy line are as defined in claim 1 with lithium, iodide in pyridine, 2,6-dimethylpyridine or 2,4,6-trimethylpyridine.

28. A process as claimed in claim 27 wherein the compound of formula (VII) is prepared by heating a compound of formula (III):

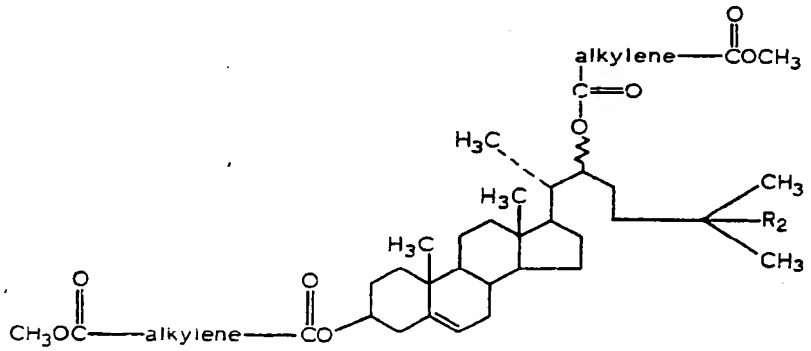


(III)

wherein R_2 and the wavy line are as defined in claim 1, with a methyl-Ω-chloro-Ω-oxoalkanoate, and separating the required product from the resulting end products by chromatography.

29. A process as claimed in claim 28 wherein the compound of formula (III) is prepared by a process as claimed in any of claims 18 to 20.

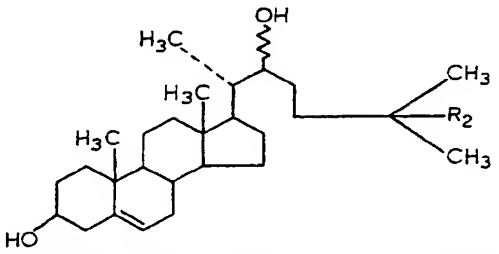
30. A process for the preparation of a compound as claimed in claim 8 which comprises heating a compound of formula (VIII):



(VIII)

wherein R_2 and the wavy line are as defined in claim 1 with lithium iodide in pyridine, 2,6-dimethylpyrindine or 2,4,6,-trimethylpyridine.

31. A process as claimed in claim 30 wherein the compound of formula (VIII) is prepared by heating a compound of formula (III):

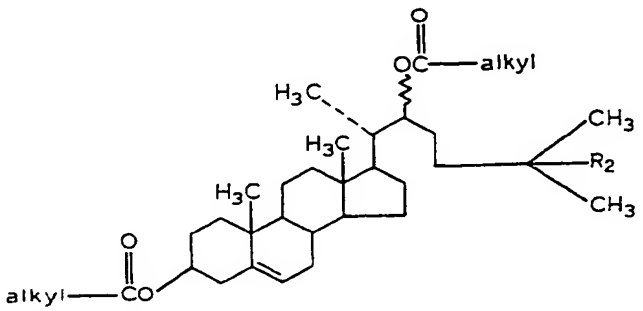


(III)

wherein R_2 and the wavy line are as defined in claim 1, with a methyl-Ω-chloro-Ω-oxoalkanoate, and separating the required product from the resulting end products by chromatography.

32. A process as claimed in claim 31, wherein the compound of formula (III) is prepared by a process as claimed in any of claims 18 to 20.

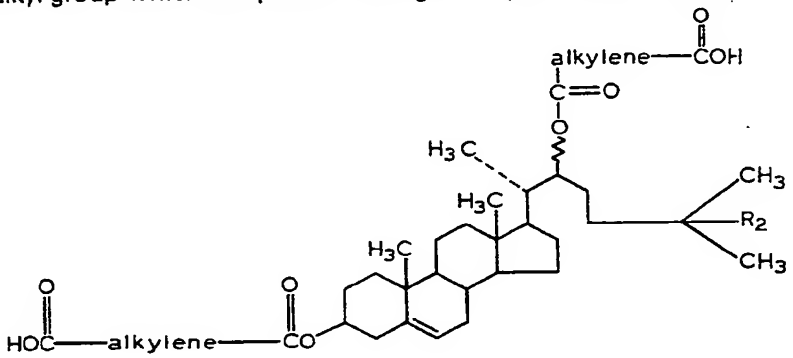
33. A process for the preparation of a compound as claimed in claim 9 wherein R_1 is a 1-oxoalkyl group which comprises heating a compound of formula (VI):



wherein R_2 and the wavy line are as defined in claim 1 with sodium bicarbonate in aqueous ethanol.

34. A process as claimed in claim 33 wherein the compound of formula (VI) is prepared by a process as claimed in claim 25 or 26.

35. A process for the preparation of a compound as claimed in claim 9 wherein R_1 is an Ω-carboxy-1-oxoalkyl group which comprises reacting a compound of formula (X):

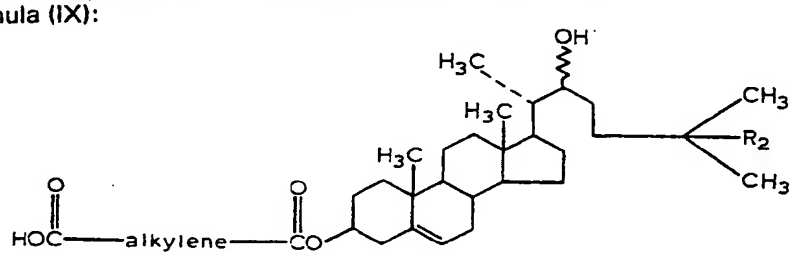


(X)

wherein R_2 and the wavy line are as defined in claim 1 with sodium bicarbonate in aqueous ethanol.

36. A process as claimed in claim 35 wherein the compound of formula (X) is prepared by a process as claimed in any of claims 30 to 32.

37. A process for the preparation of a compound as claimed in claim 10 which comprises heating a compound of formula (IX):

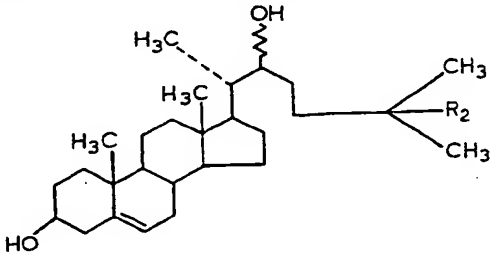


(IX)

wherein R_2 and the wavy line are as defined in claim 1 with an alkanoic acid anhydride of chloride having from 2 to 7 carbon atoms in pyridine.

38. A process as claimed in claim 37 wherein the compound of formula (IX) is prepared by a process as claimed in any of claims 27 to 29.

39. A process for the preparation of a compound as claimed in claim 7 in which R is a 3-carboxy-1-oxypropyl group which comprises heating a compound of formula (III):



(III)

wherein R_2 and the wavy line are as defined in claim 1, with butanedioc acid anhydride in pyridine.

40. A process as claimed in claim 39 wherein the compound of formulu (III) is prepared by a process as claimed in any of claims 18 to 20.

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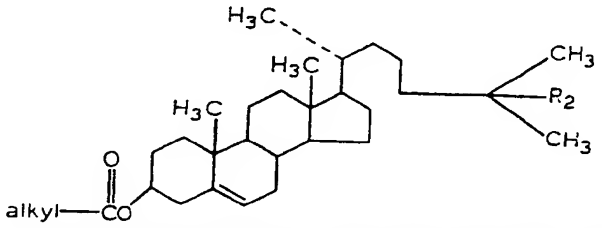
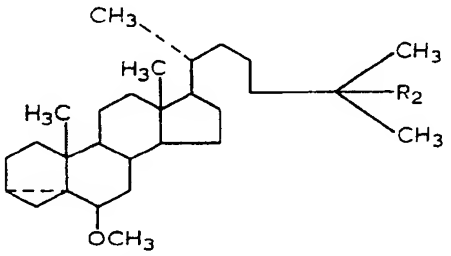
41. A process for the preparation of a compound as claimed in claim 11 which comprises contacting a compound of formula (XV):



(XV)

wherein R_2 is as defined in claim 1 with chromium oxide and pyridine in dichloromethane under an atmosphere of nitrogen.

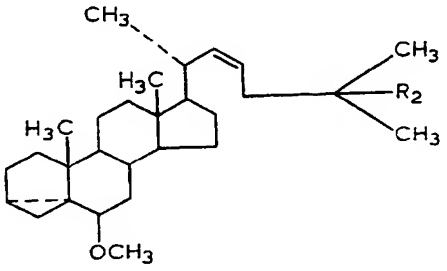
42. A process as claimed in claim 41 wherein the compound of formula (XV) is prepared by heating a compound of formula (XIV):



(XIV)

wherein R_2 is as defined in claim 1 with an alkanoic acid.

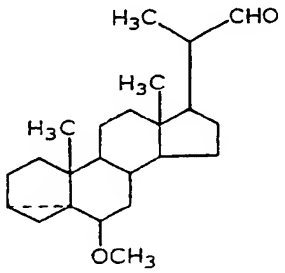
43. A process as claimed in claim 42 wherein the compound of formula (XIV) is prepared by hydrogenating a compound of formula (XIII):



(XIII)

over 5% palladium/charcoal.

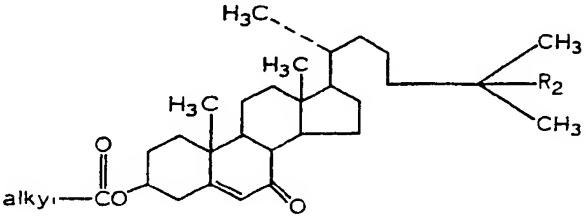
44. A process as claimed in claim 43 wherein the compound of formula (XIII) is prepared by alkylating a compound of formula (I):



(I)

with a 3,3-dimethylalkyl triphenyl phosphonium iodide.

45. A process for the preparation of a compound as claimed in claim 12 which comprises heating a compound of formula (XVI):



(XVI)

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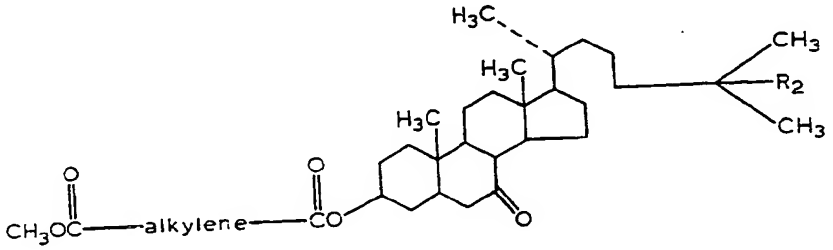
wherein R_2 is as defined in claim 1, with sodium bicarbonate in aqueous ethanol.

46. A process as claimed in claim 45 wherein the compound of formula (XVI) is prepared by a process as claimed in any of claims 41 to 44.

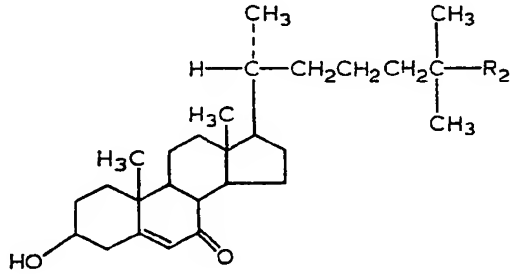
47. A process for the preparation of a compound as claimed in claim 13 which comprises heating a compound of formula (XVIII):



(XVIII)

wherein R_2 is as defined in claim 1 with lithium iodide in pyridine, 2,6-dimethylpyridine or 2,4,6-trimethylpyridine.

48. A process as claimed in claim 47 wherein the compound of formula (XVIII) is prepared by heating a compound of formula (XVII):

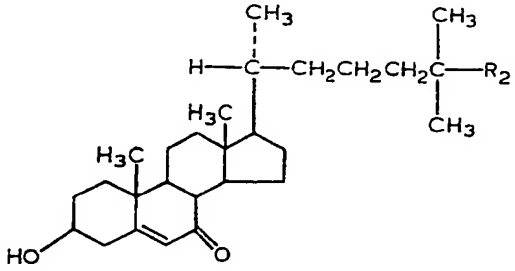


(XVII)

wherein R_2 is as defined in claim 1 with methyl Ω-chloro-Ω-oxoalkanoate in pyridine.

49. A process as claimed in claim 48 wherein the compound of formula (XVII) is prepared by a process as claimed in claim 45 or claim 46.

50. A process for the preparation of a compound as claimed in claim 13 wherein R is a 3-carboxy-1-oxopropyl group, which comprises heating a compound of formula (XVII):



(XVII)

wherein R_2 is as defined in claim 1 with butanedioic acid anhydride in pyridine.

51. A process as claimed in claim 51 wherein the compound of formula (XVII) is prepared by a process as claimed in claim 45 or claim 46.

52. A pharmaceutical formulation which comprises one or more of the compounds as claimed in any of claims 1 to 17 as active ingredient, together with a pharmaceutically acceptable carrier or diluent.

53. A pharmaceutical formulation as claimed in claim 52 in unit dose form.

54. A pharmaceutical formulation as claimed in claim 53 wherein the active ingredient is present in an amount of 500 mg.

55. A veterinary formulation which comprises one or more of the compounds as claimed in any of claims 1 to 17 together with a veterinary acceptable carrier.

56. A method of preparing a pharmaceutical formulation which comprises admixing one or more of the compounds as claimed in any of claims 1 to 17 with a pharmaceutically acceptable carrier or diluent.

57. A compound as claimed in claim 1 substantially as herein described with reference to Examples 1 to 21.

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58. A pharmaceutical formulation as claimed in any of claims 52 to 54 substantially as herein described with reference to Example 22.

59. A process as claimed in any of claims 18 to 51 substantially as herein described with reference to Examples 1 to 21.

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